

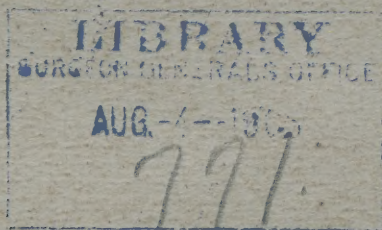
WRIGHT (J. H.)

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(MADURA FOOT)



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## A CASE OF MYCETOMA (MADURA FOOT).

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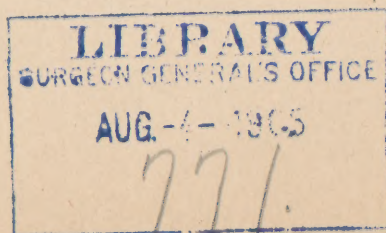
*(From the Laboratory of the Massachusetts General Hospital.)*

PLATES XXXIX AND XL.

Mycetoma is a chronic inflammatory process, most commonly affecting the tissues of the foot, in which suppurative nodular swellings, sinus formation, and enlargement and distortion of the parts are prominent features. The bones may become involved and undergo a rarefying osteitis.

The affection is characterized by the presence in the diseased tissue and in the discharge from sinuses, of peculiar granules, usually not more than one millimetre in diameter, but sometimes larger. These granules in certain cases are of a black color, of irregular shape, and are hard and rather brittle. In general they resemble grains of gunpowder. In other cases the granules are whitish, greyish or yellowish in color and are of a soft or cheesy consistency. They have been compared to fish-roe in appearance. A very few cases are also recorded in which the granules were of a red color. There is no good evidence to show that more than one kind of granules are ever present in any one case of the disease.

On account of the fact that at least two very different kinds of granules are found associated with the lesions, two varieties or forms of the disease are recognized: the "melanoid" or black variety, in which the granules are black, and the "ochroid" or pale variety, in which the granules are white to yellow in color. A. Vandyke Carter, who was the first to show that mycetoma was a special pathological process and to name the disease itself and its varieties, considered the granules to be the cause of the affection and regarded them as representing stages in the life history of one and the same fungus. Carter claimed that he had cultivated this fungus and that it grew as a pink



mould. This pink mould was studied by H. J. Berkeley and named by him "Chionyphe Carteri." The connection between this pink mould and the granules was not clearly demonstrated, owing to the conditions under which the cultivation experiments were made, so that the pink mould has not been generally believed to represent the infecting organism in the process. There can be little doubt, however, that Carter saw and recognized in the black granules the same fungus elements which have been described by later observers. This is quite apparent from an examination of the plates in his monograph,\* published in 1874.

It is now reasonably certain, however, that the organism of the black granules is of an entirely different character from the organism or organisms making up the pale or yellowish granules. Therefore in summarizing the results of various observers who have studied the nature of these granules, observations on the black granules will be considered separately from those upon the pale or yellow granules.

The disease is endemic in India and has been observed in Africa, Italy and other tropical or sub-tropical countries. In America it is of very rare occurrence, only three undoubted cases, in all, having been reported up to the present time. These are the cases of Adami and Kirkpatrick,† of Hyde, Senn and Bishop,‡ and of Pope and Lamb.§ These were all of the ochroid variety of the disease. In a case reported by Kemper and Jameson,|| the diagnosis is open to serious question, so that it is not included in the list.

*Observations on the granules of the melanoid variety.* Bristowe¶ studied the black granules in a single case. He found them to consist "of densely aggregated ramifying tubules" embedded in a dark colored matrix. They frequently showed dilatation into round or ovoid cells. These structures he considered to be elements of a fungus identical with

\* A. Vandyke Carter. On mycetoma or the fungus disease of India. London, 1874.

† *Trans. of the Assoc. of Amer. Physicians*, 1895; *Montreal Medical Journal*, January, 1896.

‡ *Journal of Cutaneous and Genito-Urinary Diseases*, xiv (1896), 1.

§ *New York Medical Journal*, September 19, 1896.

|| *American Practitioner* (Louisville), xiv (1876), 129.

¶ *Trans. Path. Soc.*, London, 1871.

that described by Carter. No cultivation experiments were made. Bristowe's description and drawings of the microscopical appearance of the granules are very satisfactory. It is quite evident that he had before him the same organism as that seen by Carter in the black granules.

Hogg \* also examined a case of the melanoid variety, finding also fungoid structures in the granules, which, to judge from his description, were evidently the same as those described by Carter and by Bristowe.

In 1888 Bassini † described a case of the black variety which occurred in Italy. The granules were found to be made up of a mycelium of filaments imbedded in a dark brown matrix. These filaments were septate, 4 to 6 millimetres in diameter, and showed irregular swellings. They had a general radiate arrangement in the granule. Besides the granules Bassini noted the presence of strings or cords made up of the same fungus filaments imbedded in a brown matrix. No spore-bearing organs were found and no cultivation experiments were made. The mycelium was regarded as representing "an imperfect vegetative form of a fungus which, in a state of perfect evolution, would be furnished with a true reproductive apparatus." Leucocytes and young blood-vessels were noted in the tissues about the granules.

Kanthack ‡ studied three cases of the melanoid variety in microscopical sections. In all of these cases he found that the black granules seemed to consist, in some instances, of a structureless, olive brown, glassy or finely granular material, in which were imbedded hollow filaments of varying calibre, having a well-marked radial arrangement. Kanthack regarded these appearances as representing degenerative changes, and sought to show that the granules were made up of an organism closely related to actinomyces, as he had found in the case of the pale granules. It is manifest from his description, however, that he saw the same fungus mycelium in the black granules which had been noted by previous observers.

The latest and most satisfactory paper on the structure and nature of the black granules is that of Boyce and Surveyor.§ They studied seven cases of the melanoid form and found in all of them that "the black particles represent a curious metamorphosis of a large, branching, septate fungus." This fungus was imbedded in or surrounded by a brown pigment substance which was readily removed by "Eau de Javelle." The

\* *Trans. Path. Soc.*, London, 1872.

† *Archiv. per le scienze mediche*, 1888, xii (1888), 309.

‡ *Journal of Path. and Bact.*, i (1893), 140.

§ *Trans. of the Royal Society of London*, 1894; *Proc. Royal Soc.*, London, 1893.

hyphæ showed great variations in the size and shape of their segments. They were branched and often disposed in a radiate manner at the periphery. No organs of fructification were observed. The fungus was not cultivated.

*Observations on the ochroid variety.* Carter's view that the pale particles represented a stage in the evolution of the same fungus as that making up the black granules has already been mentioned.

In 1870 Moxon and Hogg\* examined a case of this variety. They found that the granules showed in some instances a texture of fine fibrils, but they were unable to come to any conclusion as to their nature.

Kanthack† examined histologically twelve specimens of the ochroid variety and states that "the parasite of the ochroid variety agrees morphologically and structurally with the typical and perfect ray fungus, and there can be no reasonable doubt that both belong to the same botanical group."

Vincent‡ found that the granules in a case of the pale form of the disease were made up of a streptothrix, which grew upon potato with a marked red color. In a later paper published with Gémy,§ Vincent reports isolating in cultures the same streptothrix from a second case.

Hewlett|| examined two cases of the ochroid variety and states that the granules were very like colonies of actinomyces in structure. He considered that the process was actinomycosis.

Boyce and Surveyor¶ studied eighteen cases, and conclude that "the white particles consist largely of caseous material and of the remains of a lowly organized fungus, presenting in very many instances some of the characteristics of the fungus of actinomycosis." This organism they regarded as entirely distinct from that found in the seven specimens of the melanoid variety which they examined.

Boyce\*\* also reports having cultivated a streptothrix from a case of the ochroid variety. This streptothrix differed in its cultural peculiarities from other similar organisms.

Adami and Kirkpatrick†† conclude, from an examination of the case of the ochroid variety observed in Canada, that the granules "were

\* *Trans. Path. Soc.*, London, 1870.

† *Loc. cit.*

‡ *Annales de l'Inst. Pasteur*, viii (1894), 129.

§ *Annales de dermatologie et syphilographie*, Paris, 3<sup>me</sup> sér., t. vii, Nov., 1896.

|| *Trans. Path. Soc.*, London, 1893.

¶ *Loc. cit.*

\*\* *Hygienische Rundschau*, iv (1894), 529.

†† *Loc. cit.*

identical in general appearance with actinomyces, forming lobate, reniform masses with central dense mycelium and a radiate arrangement of filaments or clubs around the periphery. These clubs, however, were larger than those of actinomyces."

Bishop,\* in the paper published with Hyde and Senn, gives the results of his microscopical examination of a case occurring in Chicago. The granules by direct examination were made up of a dense granular central portion and a more transparent peripheral portion in which an obscure radial striation could be observed. No clubs were observed at the periphery. In sections of the tissues the granules consisted of more or less rounded masses, often having a scalloped border and occurring singly and in groups. The central portions stained faintly with hæmatoxylin. Under a high magnifying power delicate filamentous threads, having more or less of a radial arrangement, were to be made out in the masses, and, at the margin of some, delicate radiating lines. There was much to suggest actinomycosis. The tissue changes consisted essentially in the formation of granulation tissue in which giant cells were present.

From a consideration of the results of this list of observers it is quite evident that the disease known as mycetoma is due to at least two different organisms, or in other words, that the black granules of the melanoid form are composed of an organism entirely different from the organism or organisms making up the pale or ochroid granules.

There can be little doubt that the melanoid granules are composed essentially of a fungus or a hyphomycete, and that probably one and the same fungus has been seen by all observers.

The chief thing lacking in these studies of the melanoid granules has been the growing of this fungus. This cultivation has been accomplished in the case reported below.

As to the nature of the granules in the ochroid form it is difficult to form an opinion in some of the cases. It seems clear that, in most of them at least, the granules are composed of organisms of the streptothrix group, to which actinomyces is now generally thought to belong, and not to be the more highly organized hyphomycetes. That some of the cases of the ochroid variety are nothing more or less than actinomycosis seems not unlikely.

\* Loc. cit.

On account of the rarity of this disease in America, and because of the interest attaching to the parasite, the following case is reported:

*Clinical History.* The patient, an Italian woman, aged twenty-six, was admitted to the service of Dr. H. H. A. Beach, in the Massachusetts General Hospital, December 29, 1897. She was first seen in the Out-Patient Department by Dr. C. A. Porter, who recognized the condition as probably madura foot. The tissues of the left foot in the region of the base of the second and third toes on the plantar aspect presented a swollen appearance with desquamation of the epithelium. In the skin over this swollen area there was a small sinus, from which exuded on pressure a dirty, greyish fluid, containing some black, hard, irregular granules like grains of gunpowder. On the dorsum of the second toe, near its tarso-metatarsal joint, there was a small ulcer.

The disease was first noticed in July, 1897. The patient had lived in America several years. A piece of tissue was excised from the second toe for microscopical examination, and from the results of this, as well as from the appearance and character of the black granules associated with the condition, the diagnosis of mycetoma, or madura foot, of the melanoid variety was made. Amputation of the four toes, together with a part of the four corresponding metatarsal bones, was performed by Dr. Beach.

The dissection of the amputated part showed the following conditions: In the soft tissues of the plantar side of the foot near the tarso-metatarsal articulations and immediately beneath the skin was a pigeon's-egg sized, ovoid tumor nodule, sharply defined from the surrounding tissue (Plate XXXIX, Fig. 1). This nodule on section consisted of a soft, in places gelatinous, myxomatous-like tissue, traversed by an indefinite reticulum, which divided it into indefinite small areas; in these areas were imbedded black, irregular granules less than a millimetre in diameter, resembling grains of gunpowder. These granules occurred singly and in groups. In one or two places the tissue of the nodule was opaque and yellowish, as from necrosis. Two other similar nodules of small size, containing black granules, were also present. One was situated in the soft tissue of the dorsum of the foot near the base of the second and third toes; the other in the soft tissue of the first phalanx of the second toe. The larger of these nodules was about the size of a pea. The bones were not involved.

*The structure of the black granules.* Macroscopically the granules are of an irregular or mulberry shape, of a black or dark brown color, and usually less than one millimetre in diameter. They are sometimes bound together by shreds of tissue or of exudate. They are hard, rather brittle, and are not readily broken up under a cover-glass.

Microscopically they present the appearance of irregular, lobate masses of a dark brown or black opaque substance. Upon being broken up and examined with high magnifying power, little more than indefinite, brown, somewhat refringent masses can be made out. Occasionally structures suggestive of fungus may be seen, but there is little else to indicate a vegetable nature. They are very resistant to most reagents, but by soaking them for a few minutes, with or without heating, in the ordinary hospital solution of sodium hypochlorite, they lose their brown or black color and become more or less completely white and soft, according to the duration of the action of the reagent. A strong solution of potassium hydroxide also softens them to a certain extent, but does not bleach them so quickly.

Granules from the hardened specimen, bleached and softened as above outlined, when crushed and dissociated under a cover-glass, are found to consist of a mass of fungus structures together with more or less brown pigmented imbedding, or investing material, as described and figured by Boyce and Surveyor,\* and others.

Under a high magnifying power (Plate XL, Fig. 7) the greater part of the mass of the granules is seen to consist of ovoid or rounded, translucent bodies of varying sizes, closely packed together, having homogeneous interiors or containing a few refractive granules. Among these bodies typical septate, branching hyphæ may be seen, sometimes showing dilatation or varicosities of their segments. The ovoid or rounded bodies seem to be dilated or degenerated hyphal segments, for not only may they be seen in continuity with the hyphæ, but transitions between them and the hyphæ with swollen segments may be made out.

The periphery of the granules has the appearance of being made

\* Loc. cit.

up of closely set, radiating hyphæ, usually showing more or less swelling or degenerative changes in their segments.

No undoubted spore-bearing organs were observed, but occasionally a large spherical, thick walled body containing smaller rounded or ovoid or elongated refringent bodies was observed. These were regarded as representing some peculiar mode of growth or an "involution," rather than spores and spore-bearing organs.

In specimens treated with caustic potash solution, when the removal of the interstitial pigmentary substance is not complete, branched hyphal tubes may be made out imbedded in a brown refringent substance.

The test for hæmin in the black granules was negative.

In sections of the tissues, under the microscope, the granules are seen as rounded, lobate, reniform or irregular bodies of a more or less reticular structure in general (Plate XXXIX, Figs. 2 and 6, Plate XL, Fig. 8). They are deeply stained by the basic aniline dyes, but are not stained by hæmatoxylin.

In sections stained with the latter they appear to be chiefly composed of a brown-colored, somewhat hyaline substance, arranged throughout the main portion of the granules in the form of a reticulum and in indefinite or rounded clumps. This substance at the margin may merge into a narrow zone of yellow, very hyaline refractive material, in which more or less of a radial striation may sometimes be apparent.

The results of the study of the granules in sections stained in various ways may be summarized as follows:

The granules, as a rule, are made up of a central portion and a cortical portion or rind, without any definite separation between them. In the central portion are usually branching trabeculæ and rounded masses, composed of a hyaline substance, in the midst of which are numerous structures like fungus elements, cut in various planes. In some instances the central portion is made up mainly of an amorphous or indefinite material with only a few fungus hyphæ. In general the central portion is less dense in structure than the cortical portion or rind. The latter in most cases consists of a hyaline material, con-

tinuous with the hyaline trabeculae of the central portion, in which fungus elements are imbedded, sometimes arranged more or less in a radiate manner, sometimes not. The margin is irregularly scalloped or dentated. In some cases the cortical portion seems to be made up of more or less atypical hyphæ and elongated processes of hyaline substance radially disposed. In a very few instances the granules appear in the sections as irregularly shaped masses of homogeneous, hyaline or fine granular material, in which unstained or empty fungus tubules or hyphæ are embedded (Plate XL, Fig. 8).

In brief it may be said that the granules are composed of a mycelium of hyphæ or fungoid elements, more or less degenerated, embedded in or surrounded by a hyaline brown-colored refringent substance, which itself forms more or less of a reticulum.

The Gram stain and the Weigert fibrin stain, or modifications of them, stain the substance of the granules, to a variable extent depending on the amount of the decolorizing agents applied. The marginal portions of the granules hold the stain longest. In only a very few instances could the hyphæ be differentially stained by these methods.

*Cultures.* About sixty-five of the black granules were planted in various culture-media after having been washed in sterilized bouillon to remove contaminating organisms as much as possible. Nearly all of these granules were taken from freshly cut portions of the nodules of the foot after amputation. From approximately twenty-five of the sixty-five granules, a growth of a hyphomycete was obtained. The original cultures, with one or two exceptions, showed more or less contamination with the staphylococcus albus, which was abundantly present in the diseased tissue. The exceptions were in the cases of one or two grains obtained from the sinuses before the amputation and after peroxide of hydrogen had been used at intervals in the sinuses. This reagent had probably destroyed most or all of the pus cocci adherent to the grains, so that the washing in sterile bouillon sufficed to render it free from living contaminations.

The growth in all cases began from the grain and appeared after about four or five days or later. On solid culture-media it first appeared as a tuft of delicate, whitish filaments springing from the black

grain. These in the course of a few days increased in number and length, and in the case of the potato formed a dense, whitish or pale brown felt-work or membrane, having a tendency to spread widely (Plate XL, Fig. 12).

Granules planted in potato infusion, in which the contaminating cocci grew only feebly, and granules obtained free from contamination and planted in bouillon, showed after four or more days a faint greyish halo about their peripheries. In the course of some days this increased in size and was seen to consist of long, delicate, radiating filaments (Plate XL, Fig. 9), which grew in length until they reached the wall of the tube and produced the appearance of a powder puff-ball, with the granule in the centre (Plate XL, Fig. 11). Granules were readily obtained free from bacterial contamination and planted in bouillon for development, by selecting those grains which, after remaining on solid culture-media for several days, were well separated from colonies of cocci. Many of the granules, *i. e.* about forty out of the sixty-five, failed to develop any fungus. This fact bears out the idea that many of the fungus elements in the granules are degenerated, as has been mentioned.

The character of the hyphomycete may be summed up as follows:

*Morphology* (Plate XL, Figs. 8 and 10). Long branching hyphæ from 3 to 8  $\mu$  in diameter. In the younger ones delicate transverse septa are present. In the older forms there may be swellings at these points, and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall and in their interior, granules or pale areas may be seen. The branching occurs by the formation of lateral outgrowths or buds. No spore-bearing organs were observed.

*Potato.* It forms a dense, widely spreading, coherent membrane, or layer, of velvety surface, pale brown in color throughout its central portions, and white at its peripheral portions. A marked feature is the appearance of small spherical globules or droplets of dark, coffee-colored fluid on the surface of this layer. The potato takes on a dark brown color and becomes very moist (Plate XL, Fig. 12).

*Bouillon.* The growth always proceeds from the planted material,

whether grain or fragment of mycelium, in the form of fine radiating filaments, and soon produces a puff-ball appearance (Plate XL, Figs. 9, 11). Eventually the whole area of fluid is filled up with the radiating mycelium and a definite mycelial layer is formed at the surface. The medium acquires a deep coffee-brown color.

*Potato Infusion.* This was prepared by boiling about 20 grms. of potato in water, filtering and making up to 1000 cc. without neutralizing, as used by Vincent.\* In this the growth proceeds as in bouillon, except that no surface layer is formed. A peculiarity, however, noted in old cultures consists in the appearance of numerous black granules, about 1 mm. or less in diameter, in the midst of the mycelium.

These granules consist of closely packed spherical or polyhedral cells, together with some short, thick, segmented hyphæ. The walls of these cells have a black appearance and masses of them are black and opaque under the microscope. They seem to represent masses of interlacing hyphæ whose segments have been much shortened and widened, and otherwise changed.

These black masses were kindly examined by Prof. W. G. Farlow of Harvard University, who considered them to be "sclerotia."

*Agar-Agar* (plain and glucose). The growth appears as a mesh-work of widely spreading filaments, of a greyish color, on the surface of the medium. In old cultures black "sclerotia" are found as in the potato infusion. In stab cultures growth appears only at the surface.

No results were obtained from the inoculation of animals with the original granules or with cultures.

*Pathological Histology.* The tissue composing the nodules consists essentially of a formation of more or less atypical connective tissue, in various stages of development, in which foci of suppuration are present in association with the granules.

Some of the granules lie in small cavities containing polynuclear leucocytes, loose epithelioid cells and cellular detritus. These cavities may be lined either by a wall of vascular granulation tissue or by masses of epithelioid cells, together with multinucleated giant cells

\* Loc. cit.

(Plate XXXIX, Figs. 2 and 4). Other granules are closely invested by a zone of epithelioid and giant cells, and outside of this there may be an infiltration with lymphoid and plasma cells. The nodule thus formed about the granule resembles very closely a tubercle in structure. This tubercle-like formation is shown in Plate XXXIX, Figs. 5 and 6. The giant cells are often of large size and may have a peripheral arrangement of their nuclei. They are a very prominent element in the lesions.

The primary effect produced by the parasites upon the tissues seems to be the development of nodules of epithelioid cells and of giant cells from the tissues immediately about them. Later, suppurative processes occur in the nodules and abscesses are formed, which in time give rise to the development of granulation and connective tissue in large amount.

The writer is under obligations to Dr. H. H. A. Beach, of the Staff of the Massachusetts General Hospital, and to Prof. W. G. Farlow, of Harvard University, for courtesies shown him in connection with the study of this case.

The photographs which accompany this paper were made by Mr. Louis F. Brown. For valuable instruction and suggestions in their preparation the writer is greatly indebted to Mr. John G. Hubbard, of Brookline, Mass.

#### DESCRIPTION OF PLATES XXXIX AND XL.

All of the figures are photomicrographs from original specimens.

##### PLATE XXXIX.

Fig. 1. Portion of amputated part showing the black granules and general character of the lesions as seen with naked eye.

Fig. 2. Section showing black granules and general features of the lesions as they appear under a low magnifying power—Zeiss  $a_2$ .

Figs. 3, 4, 5 and 6. Sections showing the granules and surrounding cells. All, with the exception of Fig. 4, taken with Zeiss apochromat. 16 mm. Fig. 4 taken with Zeiss apochromat. 4 mm. Figs. 5 and 6 represent the tubercle-like nodules, with the parasite in the centre, as described in the text.

##### PLATE XL.

Fig. 7. Fungus elements from a granule after bleaching and softening with sodium hypochlorite solution. A branching, septate hypha is shown. Zeiss apochromat. 4 mm.

Fig. 8. Section showing margin of two contiguous granules, highly magnified. Zeiss apochromat. 2 mm.

The small clearer areas represent fungoid elements. The darker substance represents the hyaline imbedding substance.

Fig. 9. Black granule with mycelial growth proceeding from it. Zeiss a. Culture in bouillon.

Fig. 10. Showing structure and appearances of the hyphæ of the mycelium obtained from the granules. Zeiss apochromat. 4 mm.

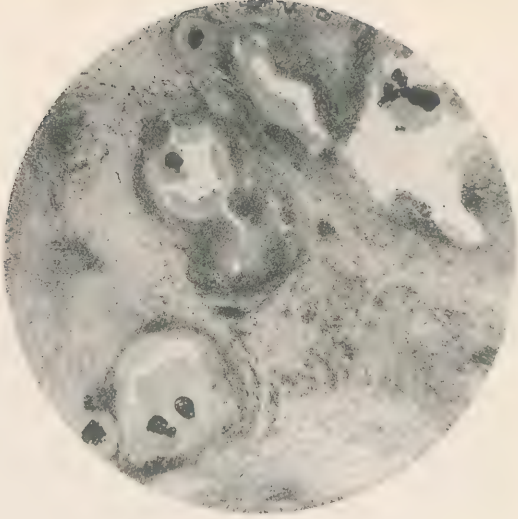
Fig. 11. Two bouillon cultures showing the powder puff-ball appearance. In one the black granule is seen in the centre of the growth.

Fig. 12. Potato culture of the hyphomycete obtained from the granules. The black globules are composed of a dark brown fluid.

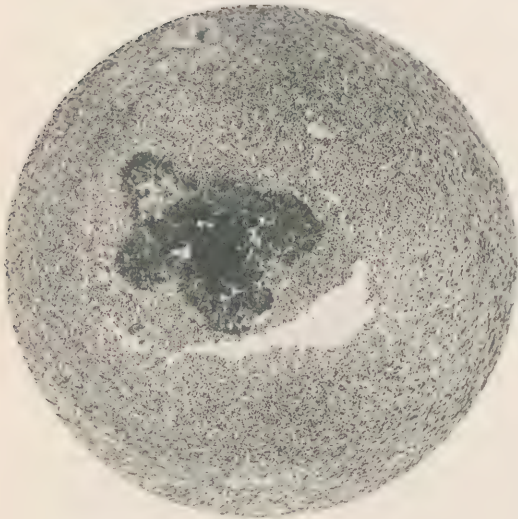




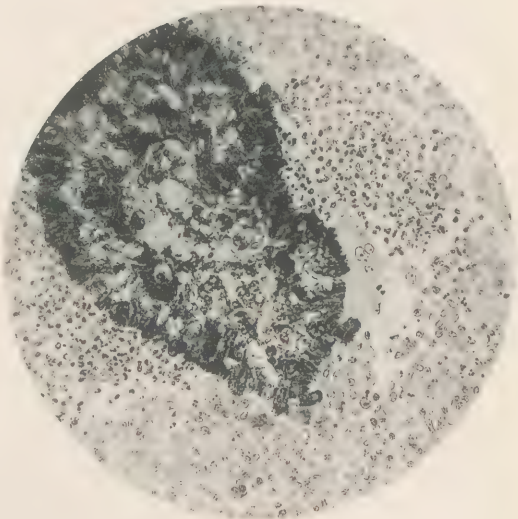
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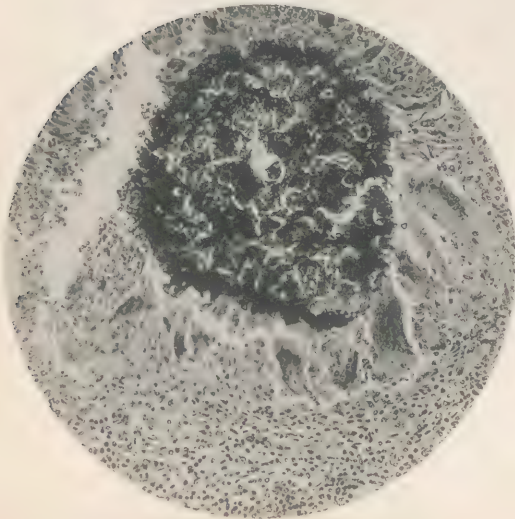
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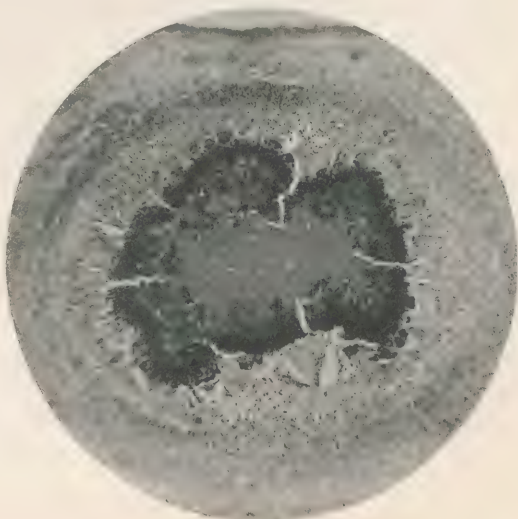
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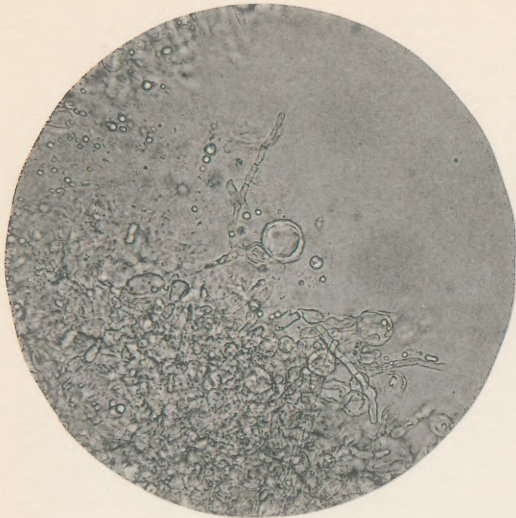


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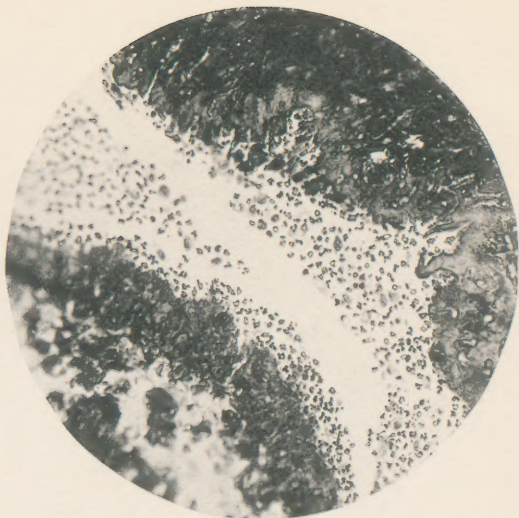


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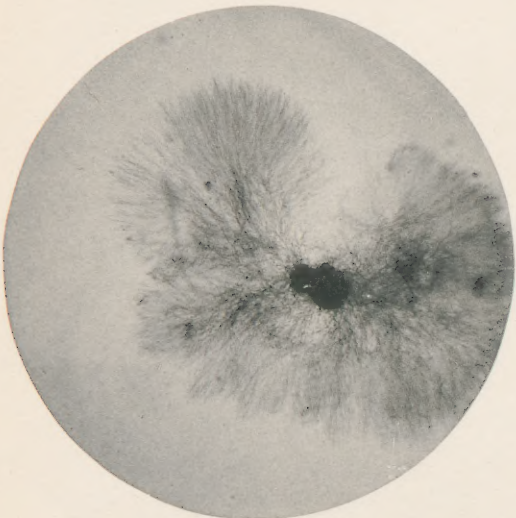




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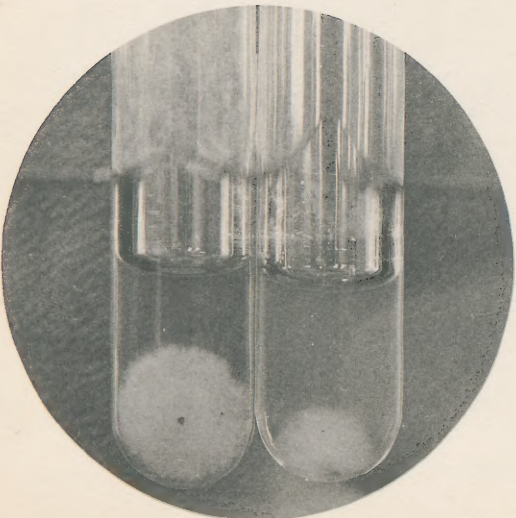
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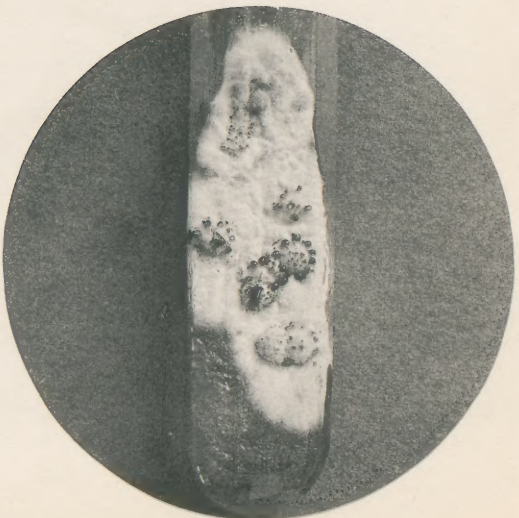
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